

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44bis)

Applicant's or agent's file reference 0000055443	FOR FURTHER ACTION	See item 4 below
International application No. PCT/EP2005/002734	International filing date (<i>day/month/year</i>) 15 March 2005 (15.03.2005)	Priority date (<i>day/month/year</i>) 17 March 2004 (17.03.2004)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant BASF PLANT SCIENCE GmbH		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 bis.1(a).
 2. This REPORT consists of a total of 7 sheets, including this cover sheet.
- In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

- | | | |
|-------------------------------------|--------------|---|
| <input checked="" type="checkbox"/> | Box No. I | Basis of the report |
| <input type="checkbox"/> | Box No. II | Priority |
| <input type="checkbox"/> | Box No. III | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| <input type="checkbox"/> | Box No. IV | Lack of unity of invention |
| <input checked="" type="checkbox"/> | Box No. V | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| <input type="checkbox"/> | Box No. VI | Certain documents cited |
| <input type="checkbox"/> | Box No. VII | Certain defects in the international application |
| <input checked="" type="checkbox"/> | Box No. VIII | Certain observations on the international application |

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44bis.3(c) and 93bis.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44bis.2).

<p style="text-align: center;">The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland</p> <p>Facsimile No. +41 22 338 82 70</p>	<p>Date of issuance of this report 19 September 2006 (19.09.2006)</p> <p>Authorized officer Agnes Wittmann-Regis</p> <p>e-mail: pt06@wipo.int</p>
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From the
INTERNATIONAL SEARCHING AUTHORITY

see form PCT/ISA/220

PCT

REC'D 06 JUL 2005

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/SA210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.
PCT/EP2005/002734

International filing date (day/month/year)
15.03.2005

Priority date (day/month/year)
17.03.2004

International Patent Classification (IPC) or both national classification and IPC
C12N15/82

Applicant
BASF PLANT SCIENCE GMBH

1. This opinion contains indications relating to the following items:

- | | |
|--|--|
| <input checked="" type="checkbox"/> Box No. I | Basis of the opinion |
| <input type="checkbox"/> Box No. II | Priority |
| <input type="checkbox"/> Box No. III | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| <input type="checkbox"/> Box No. IV | Lack of unity of invention |
| <input checked="" type="checkbox"/> Box No. V | Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| <input type="checkbox"/> Box No. VI | Certain documents cited |
| <input type="checkbox"/> Box No. VII | Certain defects in the international application |
| <input checked="" type="checkbox"/> Box No. VIII | Certain observations on the international application |

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/SA/220.

3. For further details, see notes to Form PCT/ISA/220.

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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/EP2005/002734

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
 - ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
 - ☒ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material:
 - ☒ in written format
 - ☒ in computer readable form
 - c. time of filing/furnishing:
 - ☒ contained in the international application as filed.
 - ☒ filed together with the international application in computer readable form.
 - ☐ furnished subsequently to this Authority for the purposes of search.
3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/EP2005/002734

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-26
	No: Claims	
Inventive step (IS)	Yes: Claims	
	No: Claims	1-26
Industrial applicability (IA)	Yes: Claims	1-26
	No: Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

**Reasoned statement with regard to novelty, inventive step or industrial applicability;
citations and explanations supporting such statements.**

1. The present application refers to a method for producing transgenic plant comprising the use of a sequence encoding a D-amino acid oxidase as both positive and negative selection marker. The application also refers to the constructs to be used in this method, to vectors, transformed cells and transformed organisms.
2. Reference is made to the following documents :
 - D1: WO 03/060133 A (SWETREE TECHNOLOGIES AB ; ERIKSON OSKAR (SE); NAESHOLM TORGNY (SE); HE) 24 July 2003 (2003-07-24)
 - D2: WO 03/004659 A (BIESGEN CHRISTIAN ; PUCHTA HOLGER (DE); INST F PFLANZENGENETIK (DE); S) 16 January 2003 (2003-01-16)
 - D3: ERIKSON OSKAR ET AL: "A conditional marker gene allowing both positive and negative selection in plants." NATURE BIOTECHNOLOGY. APR 2004, vol. 22, no. 4, April 2004 (2004-04), pages 455-458.
 - D4: DATABASE UNIPROT D-amino acid oxidase. Candida Boidinii. 1 March 2001 (2001-03-01), "DAO1" retrieved from EBI Database accession no. Q9HGY3
 - D5: DATABASE UNIPROT putative D-amino acid oxidase. S. coelicolor 1 November 1999 (1999-11-01), retrieved from EBI Database accession no. Q9X7P6
 - D6: DATABASE UNIPROT SPCC1450.07c protein. Schizosaccharomices pombe 1 November 1999 (1999-11-01), retrieved from EBI Database accession no. Q9Y7N4
 - D7: DATABASE UNIPROT D-amino acid oxidase. Trigonopsis variabilis 1 November 1997 (1997-11-01), retrieved from EBI Database accession no. OXDA_TRIVR
 - D8: DATABASE UNIPROT D-amino acid oxidase. Fusarium solani 1 March 1992 (1992-03-01), retrieved from EBI Database accession no. OXDA_FUSSO
 - D9: DATABASE UNIPROT D-amino acid oxidase. Rhodosporidium toruloides 1 November 1995 (1995-11-01), retrieved from EBI Database accession no. OXDA_RHOTO
 - D10: DATABASE UNIPROT Putative D-amino acid oxidase. C. elegans (1997-

11-01), retrieved from EBI Database accession no. OXDA_CAEEL

3. Lack of inventive step; Article 33(3) PCT.

In the prior art, numerous methods for obtaining marker free transgenic plants are disclosed (see D2 for example). These methods imply the use of both one positive selection marker (for the selection of the transformed cells) and one negative selection marker (for the selection of marker-free cells or plants).

In view of this prior art, the problem to be solved by the present application can be seen as the provision of an improved marker system for the generation of marker free plants.

The application solves this problem by the provision of a gene encoding a D-amino acid oxidase which can be used both as a positive or negative selection marker instead of the two distinct markers used in the prior art.

Document D1 discloses the transformation of *A. thaliana* with a gene encoding a D-amino acid oxidase. The D-amino acid oxidase disclosed in D1 can be used as both a positive (p. 35, lines 18-22) or a negative marker (p. 35, line 24 to p. 36, line 6) depending on the D-amino acid present in the culture medium.

The International Search Authority (ISA) is of the opinion that the skilled person, searching for an alternative to the well-known positive + negative marker system would have needed no inventive activity to contemplate using the dual marker disclosed in D1 which has been shown to be functional in *Arabidopsis thaliana*. Moreover, the skilled person was well aware of the advantage of having one single gene to be removed rather than two genes.

The sequences of the D-amino acid oxidases used in the present application were already known (see D4-D10).

Therefore, claims 1-26 are considered to lack inventive step in the sense of Article 33(3) PCT.

Re Item VIII

Certain observations on the international application

1. Claim 1 (v) refer to the step of breaking the combination between the first expression cassette and the second expression cassette. This wording is vague and renders the scope of the claim unclear. Which combination should be broken and how ? (Article 6 PCT)
2. Claim 5 and 6 refer to derivatives of D-amino-acids. These derivatives are not defined at all what renders the scope of the claims unclear (Article 6 PCT).
3. The wording "has an enzymatic activity **against...**" (bold added) used in claim 13 is unclear (Article 6 PCT).
4. Claim 19 refers, inter alia, to chimeras of the homing endonucleases I-SceI, I-CpaI, I-CpaiI, I-CreI and I-ChuI. These chimeras are not characterized by any technical features what renders the scope of claim 19 unclear (Article 6 PCT).
5. Claim 25 refers to general cells and non-human organisms comprising the DNA construct of any of claims 11 to 21 or the vector of claim 22. The attention of the applicant is drawn to the fact that the only organisms disclosed in the present application are plants. Therefore, the subject-matter of claim 25 could be considered as not supported by the description of the present application as far as it refers to other non-human organisms (article 5 PCT in combination with article 6 PCT).